(12) UK Patent Application (19) GB (11) 2 075 376 A

(21) Application No 8113722
(22) Date of filing 5 May 1981
(30) Priority data
(31) 146461
146462
(32) 5 May 1980
(33) United States of America (US)
(43) Application published 18 Nov 1981
(51) INT CL³ B04B 7/00 5/04
(52) Domestic classification B2P 10A
(56) Documents cited None
(58) Field of search B2P
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(54) **Process and device for centrifugal separation of platelets**

(57) This invention relates to a method and apparatus for the separation of finely divided solid particles dissimilar in size and/or density in a liquid, such as platelets and other blood cells in a blood sample. The separation of, for instance, platelets is accomplished by subjecting the blood sample to centrifugal force in a rotatable chamber while displacing the platelets from the blood sample by injecting a relatively small volume of saline into the centrifugally outer end of the chamber. In a preferred apparatus, the saline is injected via a port (15) into the blood sample by driving a chamber (14) supporting the blood sample into a saline filled cavity under the influence of centrifugal force. The chamber (14) is formed in a piston (12) slidable in a cylinder (13) which in use is placed in the swinging bucket of a centrifuge.

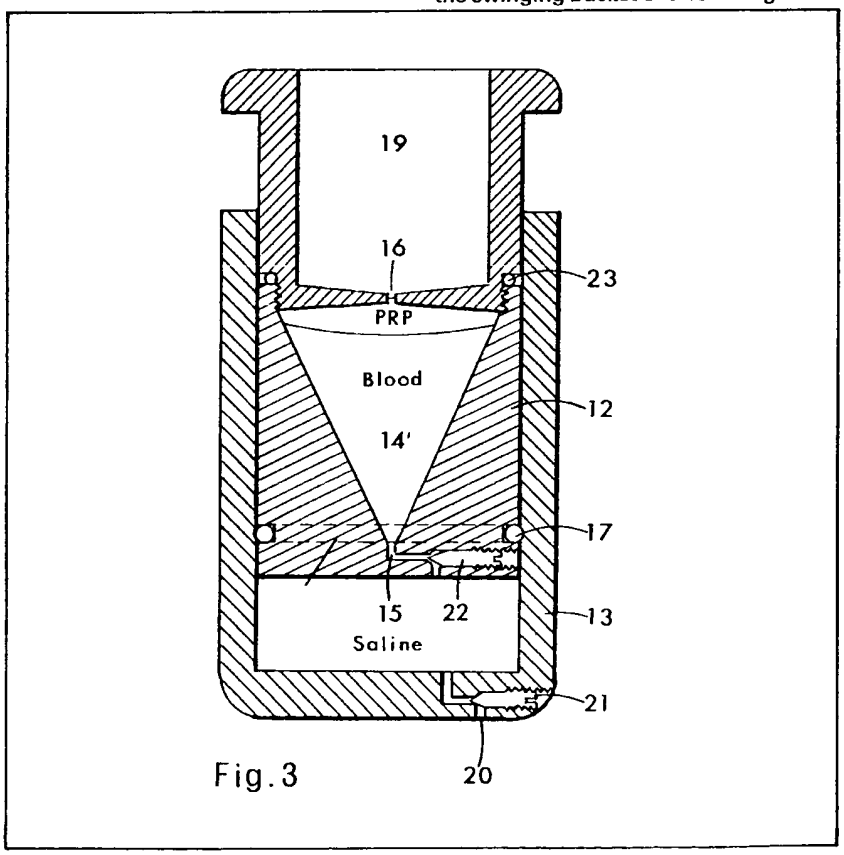

Fig.3

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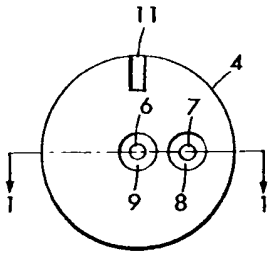

Fig.1

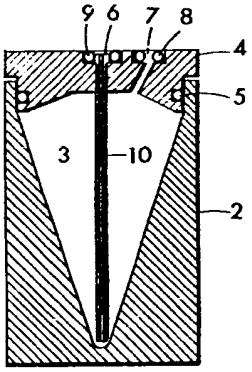

Fig.2

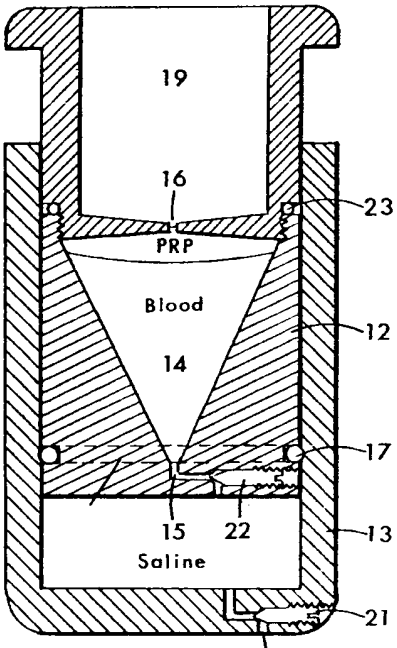

Fig.3

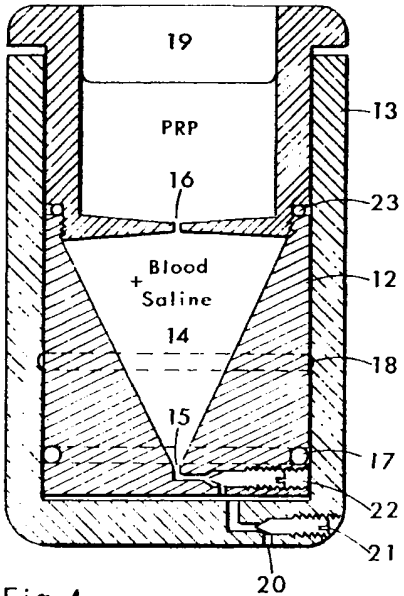

Fig.4

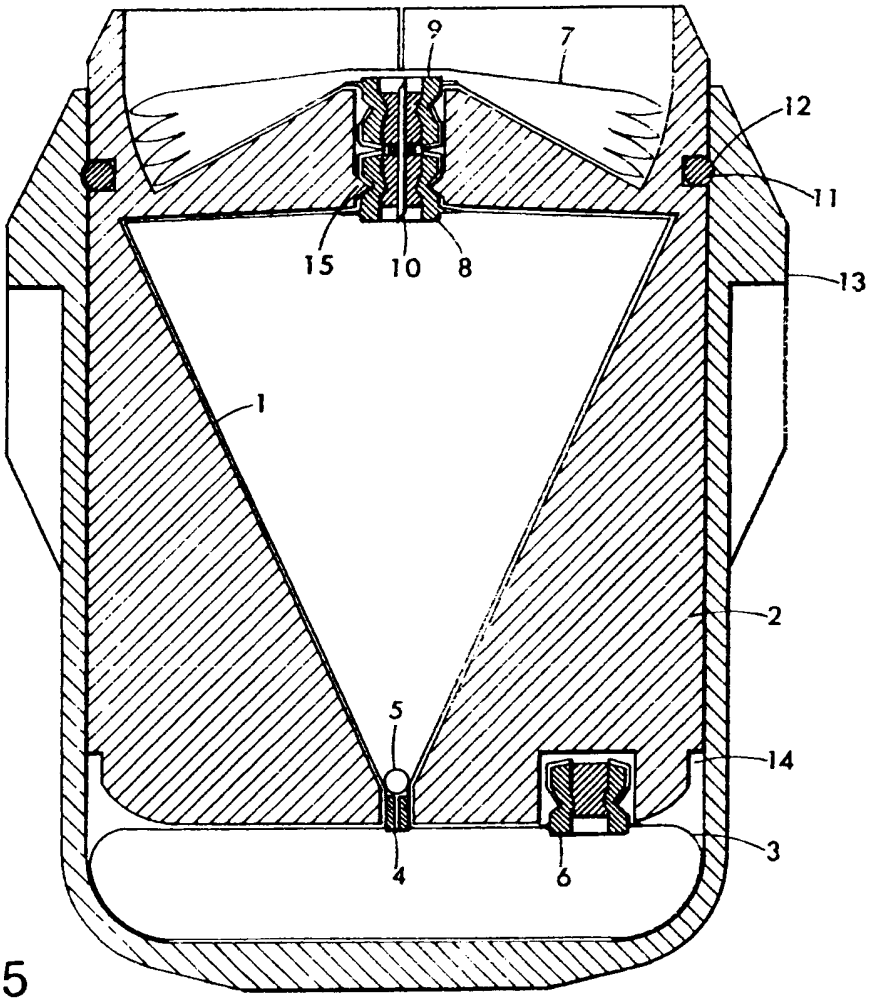

Fig. 5

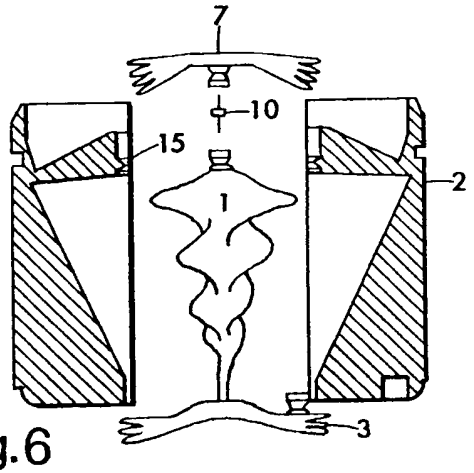

Fig.6

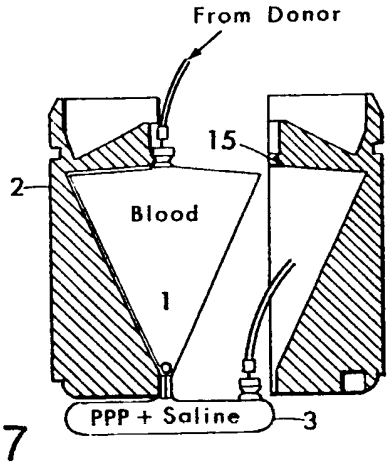

Fig.7

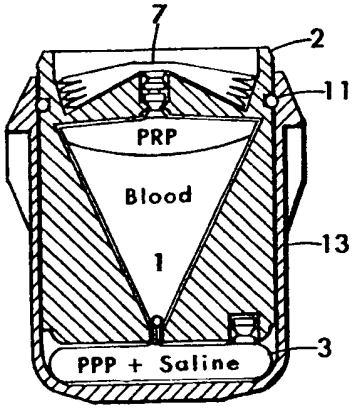

Fig.8

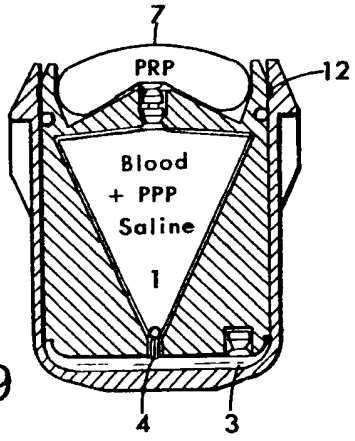

Fig.9

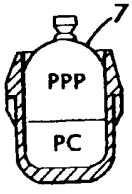

Fig.10

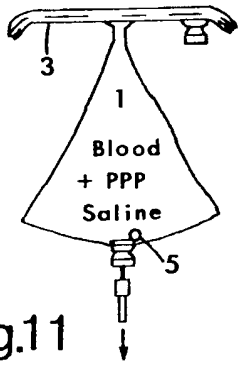

Fig.11

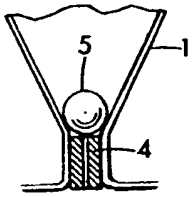

Fig.12

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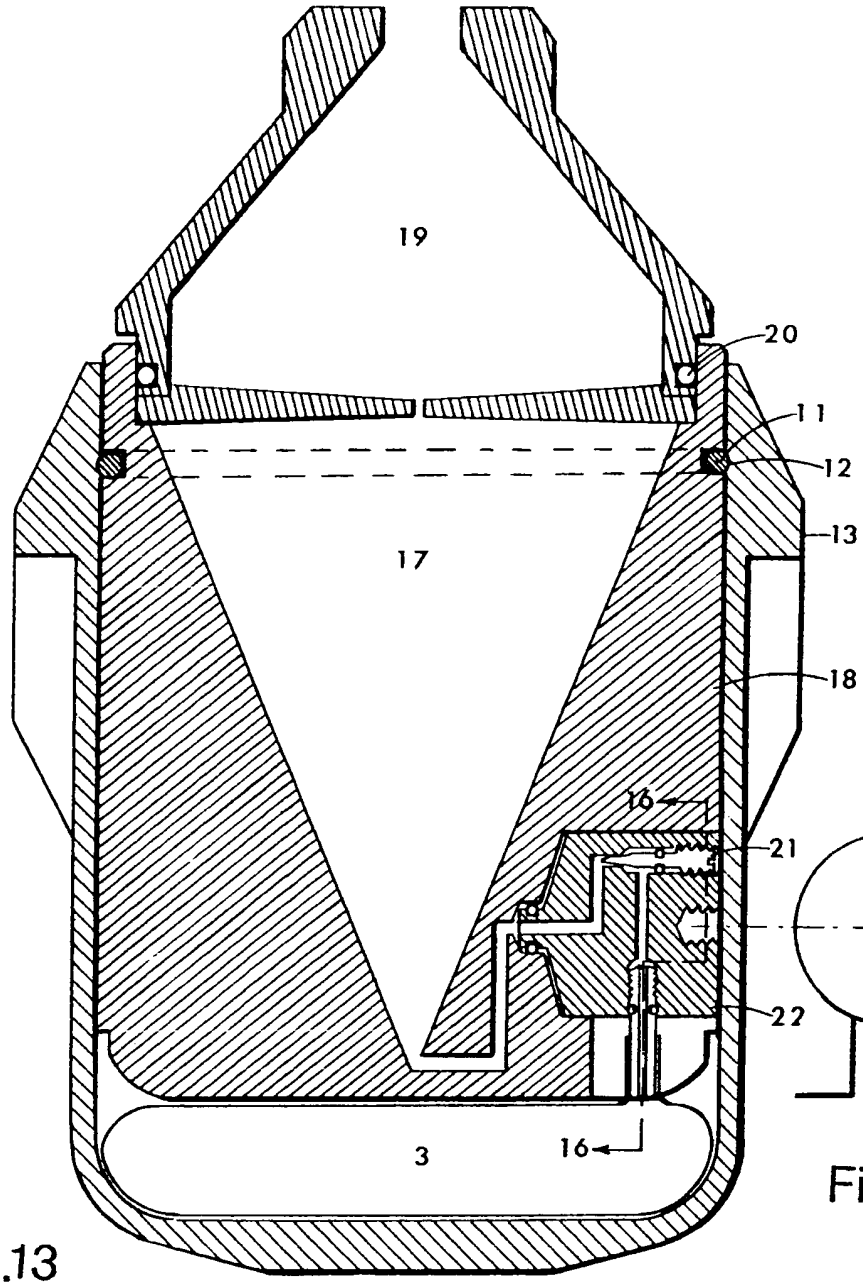

Fig.13

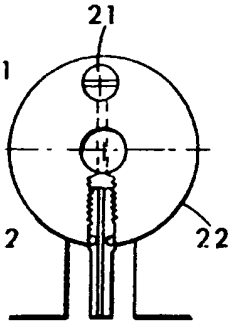

Fig.14

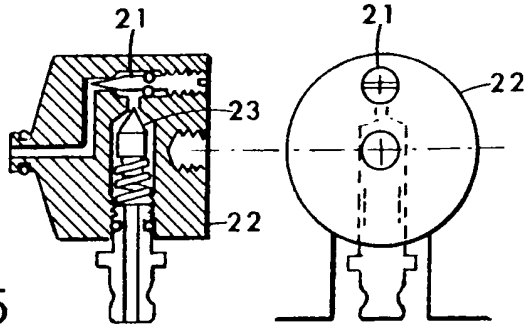

Fig.15

SPECIFICATION

Process and device for centrifugal separation of platelets

This invention pertains broadly to centrifugal separation of finely divided solid particles. More particularly, the invention relates to a new process and a device for centrifugal separation of solid particles dissimilar in size and/or density, such as platelets and other blood cells or various types of synthetic particles and beads.

Transfusion of platelets is now a widely used form of therapy for the treatment of haemorrhage in thrombocytopenic and thrombocytopathic patients. It is indispensable in the protocol treatment programs for acute leukaemia, apalstic anaemia, platelet deficiency, as well as replacement therapy in major surgical procedures.

Platelets are separated from whole blood as platelet-rich plasma (PRP) by procedures involving centrifugation. Transfusion of platelets is accomplished most often in a form of platelet-concentrates (PC) which are prepared by the high speed centrifugation of PRP. Several units of blood are required to obtain a sufficient number of platelets for therapeutic effectiveness. The use of multiple donors increases risks of iso-immunization and transmission of disease. In order to procure enough platelets from a single donor, the technique of plasma-pheresis is required in which platelet-poor plasma (PPP) and packed red blood cells (RBC) are returned to the donor's blood circulation. The commonly used procedure of slow speed centrifugation with the routine equipment found in any blood bank is cumbersome, very time-consuming, and yields platelets heavily contaminated with white blood cells (WBC) and RBCs and gave low platelet recovery. A considerable improvement in the procurement of platelets has been made by the use of specialized blood processing equipment, Haemonetics-30, which permits the collection of two to four units of PC from a single donor. In this procedure, known as plateletpheresis, blood is pumped directly from the donor through a rotary seal into a centrifuge plastic bowl. Blood components are separated by forming concentric bands which overflow from the bowl in a sequence, depending on their specific density. This technique, however, permits one to harvest on the average only 46 percent of platelets from the blood circulating in the bowl. In order to collect the 4×10^{11} platelets required for therapeutically effective transfusion, at least 6 litres of blood must be processed, which takes from 2 to 3 hours of the donor's time. The operation is time-consuming and costly. Also, large initial investment for purchasing expensive equipment is required. Collected with this technique, platelets are heavily contaminated with WBCs and RBCs. Administration of PC contaminated with WBC may cause serious complications in patients. Platelets and WBC share HL-A antigens which are more immunogenic on WBC than those on platelets. As a result, WBC contamination may be responsible for the alloimmunization in patients which, in turn, causes with each consecutive transfusion progressive reduction in hemostatic effectiveness of platelets and, in more severe cases, may lead to post-transfusion thrombocytyopenia. There are also systemic reactions which may occur within 20 minutes after completion of a platelet transfusion consisting of chills and fever. Antibodies to contaminating leucocytes are implicated in these reactions. Because of these side effects, removal of contaminating WBCs from PC by differential centrifugation is strongly recommended, which further complicates the procedure. Clearly, there is a need for improvement of platelet collection technique by making it safer and more effective in collecting PC at a higher yield, free of WBC and involving shorter time and lower cost than the present techniques offered.

The art of separating finely divided dissimilar solid particles is largely based on differential sedimentation by centrifugation. The main problems inherent in this process are partial trapping of small particles by large ones and a lack of a sharp end point of separation during sedimentation. This confronts the users with a compromising situation where in order to increase the yield of separated particles, their purity will decrease and vice versa.

Bearing the above in mind, it is an object of the present invention to provide a new process and a device for the separation of finely divided solid particles dissimilar in size and/or density while allowing both maximum yield and purity of the separated particles. In order to accomplish this object in one embodiment of a method in accordance with this invention, the existing process and system known as counterflow centrifugation (CC) or centrifugal elutriation (CE) have been substantially modified to adapt both of them for this new use.

In the CE process and system, developed by Beckman Company, the separation of particles is accomplished in a separation chamber within the Elutriator rotor. The tendency of particles to sediment in a centrifugal field is balanced in the chamber by a liquid flow in the opposite direction. By increasing the flow rate, smaller particles are washed out while larger or denser particles remain in the chamber. To accomplish the separation of particles, a large volume of liquid is made to pass through the separation chamber at a relatively high flow rate. Pumping of liquid into the rotor and through the separation chamber is accomplished by means of a rotary seal. Although both the Beckman system and their procedure are being successfully used for the separation of such blood cells as granulocytes, monocytes and young red cells, both of them are not suitable for the separation of platelets.

In accordance with this invention therefore we provide a method of separating platelets from a blood sample comprising: supporting the blood sample in a centrifugal chamber having inner and outer ends, subjecting a chamber to an initial cen-

trifugal force of at least a predetermined minimum to stratify red cells in the sample away from the inner end, and subjecting the chamber to a second centrifugal force while injecting into the outer end of the chamber from a cavity a displacing liquid having a volume of between 0.2 and two times the volume of plasma in the sample at a flow rate less than about 1.0 ml. per minute per ml. of the blood sample, and displacing platelet rich plasma from the inner end of the chamber at substantially the same volume and rate, with the displacing liquid ejected from the cavity by the second centrifugal force on the blood sample. Based on an experimental study of chambers with different configurations, the one with a conical shape and larger in volume than the Beckman chamber, being 10.5 ml instead of 4.5 ml, was found to be most suitable for platelet separation. The reasons for this are that the conical chamber with about a 40° angle at its taper provides a steep and uniform fluid velocity gradient, directed against the centrifugal force gradient, thereby allowing one to keep blood cells in the chamber at a steady state equilibrium as a dense cell suspension with a sharp upper boundary which bears physical characteristics of a fluidized bed of particles. This fluidized bed of blood cells acts as a depth filter allowing platelets to pass through freely while retaining all other blood cells in the chamber. An important difference in this procedure from that of Beckman is that a very low flow rate of 3.5 ml/min and a small volume of medium of 5 to 8 ml are required for the separation of platelets, while for the separation of other blood cells by Beckman's CE procedure, flow rates from 15 to 25 ml/min and volumes of medium from 700 to 1,000 ml are required. Also, separation of platelets by this process requires about two minutes for its completion, while the separation of other blood cells requires from 40 to 60 minutes. As a result, the separation process in the present invention can be characterized as a displacement process combined with the filtration in which a small volume of saline rapidly displaces PRP from whole blood. In contrast, cell separation by the CE process involves velocity sedimentation by washing with large volumes of medium. The supporting experimental evidence for the existence of the filtration action in such a fluidized bed of blood cells contained in the separation chamber is provided by unsuccessful attempts to remove the RBCs and WBCs contaminations from the PRP preparations collected by the Haemonetics-30 blood process. Apparently, presence of a much lower number of blood cells in this PRP preparation, as compared to that with whole blood, did not permit attainment of the self-stabilizing and filtering action by the fluidized bed of blood cells, which resulted in the displacement of a large number of RBCs and WBCs together with PRP.

For effective separation of PRP from whole blood, the separation chamber is preloaded with blood outside the rotor rather than loading the chamber during centrifugation as in Beckman's procedure. Beckman's procedure for the loading of blood was found totally inadequate for the separation of platelets because it frequently caused packing of blood cells, resulting in blood hemolysis accompanied by release of ADP. The latter apparently causes platelets to aggregate which, in turn, prevents their separation. By preloading the chamber with blood this problem is completely eliminated.

A further advantage obtained with the method in accordance with this invention is the initial clearance at the top of the chamber from blood cells by centrifugation for one minute without starting the counterflow of saline. This prevents an immediate elution of blood cells from the top of the chamber which would otherwise contaminate the exit line and prevent the collection of pure PRP.

Another object of this invention is to provide a self-contained system for the separation of dissilimar solid particles without involving the use of the Beckman Elutriator rotor.

We therefore provide apparatus for separating solid particles such as platelets from a liquid sample such as blood comprising a body adapted to be subjected to centrifugal force and containing a cavity adapted to receive a volume of displacing liquid therein, a piston mounted in the body for movement into the cavity in response to centrifugal force on the piston with the piston containing a centrifugal chamber for containing a sample from which particles are to be separated, and having inner and outer ends, and injection passageways means to allow movement of displacement liquid from the cavity to the centrifugal outer end of the chamber in response to movement of the piston into the cavity, a discharge passageway at the centrifugally inner end of the chamber for discharging particle rich liquid in response to injection of displacing liquid into the chamber, and means for preventing flow of displacing liquid from cavity to the chamber before centrifugal stratification of blood cells in the chamber.

In preferred embodiments of apparatus according to this invention to be described in detail hereinafter separation of dissimilar solid particles, such as platelets and other blood cells, is effected by holding red and white blood cells with the aid of centrifugal force at a steady state equilibrium in a chamber, preferably of a conical shape, while displacing platelet rich plasma (PRP) with an equivalent volume of normal saline which is filtering through the suspension of blood cells in the direction generally against the centrifugal force. The liquid medium enters the conical chamber at its vertex which is oriented away from the centre of rotation, thereby being at the centrifugal outer end of the chamber, while PRP exits the chamber through its base, which is the centrifugal inner end of the chamber. The liquid flow is generated by means of a piston pump in response to centrifugal force. The apparatus is designed as an insert to be used in a centrifuge swinging bucket, and consists of a cylinder and a piston, the latter incorporating both the conical separation chamber at its lower end and the PRP receiving chamber at its upper end. There is a needle valve in the piston controlling the flow rate of the medium flowing from the cylinder through the passageway leading to the conical chamber's centrifugal outer end. To prevent an immediate discharge of blood cells from the conical chamber by the flow of saline at the start of centrifugation, a control means is provided for holding

movement of the piston into the cylinder until a maximum centrifugal force is applied. The control means comprises an o-ring on the piston received in a groove in the wall of the cylinder. This provision allows one to clear blood cells from the centripetal end of the chamber by sedimenting cells at a low centrifugal speed applied for one minute after which centrifugal speed is raised to a required higher value at which the o-ring snaps from the groove and the piston begins to force medium from the cylinder into the conical chamber.

A self-contained system, as described above, for the effective collection of PRP from whole blood, preferably from the same donor, free of other blood cells and in a quanity and quality compatible with the requirements practised in blood banks and in hospitals is described which provides a totally sealed, disposable, low-cost system consisting of three interconnected collapsible plastic bags held together in a rigid support which is fitted into a centrifuge bucket. Preferably the three bags comprise a middle bag, preferably conical in shape, filled with blood and functioning as a separation chamber; a lower bag holding normal saline for displacement of PRP from the middle bag; and an upper bag for receiving PRP displaced from the middle bag. The system is made sterile and incorporates means for aseptic handling during all the procedural steps.

Also means are provided for delaying the start of the fluid flow during the beginning of centrifugation for about a minute in order to allow initial clearance at the top of the chamber from red and white blood cells as described above. This is accomplished either by an elastic o-ring fixed around the plunger and a groove on the inner wall of the cylinder which retains the o-ring in place until the centrifugal force reaches a certain prescribed value, or by installing a spring-loaded valve which will open only at a certain prescribed g-force.

Referring now to the accompanying drawings:

Fig. 1 is an upper plan view of a separation chamber.

Fig. 2 is a vertical section of the separation chamber taken along the line 1-1 in Fig. 1.

Fig. 3 is a vertical section of one embodiment of apparatus for a self contained system taken along the vertical central axis and showing the plunger in the upper position.

Fig. 4 is a vertical section of the apparatus shown in Fig. 3 taken along the vertical central axis and showing the plunger in the lower position.

Fig. 5 is a vertical section of the scaled-up version of a second embodiment of apparatus for a self contained system taken along the vertical central axis and showing the apparatus consisting of three collapsible bags inserted into the centrifuge bucket.

Fig. 6 is a vertical section of the apparatus shown in Fig. 5 taken along the vertical central axis and showing the apparatus in disassembled form and with empty bags.

Fig. 7 is a vertical section of the apparatus shown in Fig. 5 taken along the vertical central axis and showing the apparatus in a partially assembled form and having two bags filled.

Fig. 8 is a vertical section of the apparatus shown in Fig. 5 taken along the vertical central axis and showing the apparatus being inserted into the centrifuge bucket and locked at the upper position in the bucket.

Fig. 9 is a vertical section of the apparatus shown in Fig. 5 taken along the vertical central axis and showing the apparatus at the lower position in the centrifuge bucket.

Fig. 10 is a vertical section of the centrifuge bucket containing an inserted bag taken along the vertical central axis and showing separation of PPP and PC in the bag.

Fig. 11 is a vertical section of the conical bag together with its satellite bag taken along the vertical central axis and showing both bags in the inverted position with the blood reinfusion line connected.

Fig. 12 is an enlarged fragmentary vertical central section through the capillary inlet port and a ball valve at the bottom of the conical bag.

Fig. 13 is a vertical section of another embodiment of a platelets separation device taken along the vertical central axis and showing means for regulating flow rate and for disconnecting collapsible bag.

Fig. 14 is a fragmentary cross-section through a modular insert incorporating a needle valve for the regulation of flow rate and a connecting nipple, and taken along the lines 16 and 16 shown in Fig. 13.

Fig. 15 is a fragmentary cross-section through a modular insert incorporating a needle valve for regulating flow rate and the spring loaded valve activated by centrifugal force.

The device shown in Fig. 1 and 2 represents the separation chamber made to operate in the Beckman JE-6 Elutriator rotor and the Beckman J-21 preparative centrifuge. The main body of the chamber 2 has a conical cavity 3 and a lid 4 sealed with the o-ring 5. The lid has two ports, the central one 6 is the inlet port and the off-centre one 7 is the outlet port. Both ports are sealed with the o-rings 8 and 9. Through the central port 6 a tube 10 is inserted which opens at a close proximity from the bottom of the chamber 3. The lid has a notch 11 which serves to locate the proper position of the chamber in the Beckman Elutriator rotor.

The separation chamber shown in Fig. 1 and 2 represents an optimal design for the separation of platelets from whole blood. This design has been arrived at after an extensive experimental testing of many chambers of different geometrical shapes, including several conical ones with different angles at their taper, parabolic chambers with and without flare at their broad end, as well as the Beckman chamber. The latter one was found completely ineffective for the separation of platelets. The centrifugal elutriation procedure developed by the Beckman Company for the separation of different cells and particles was also found unsuitable for the above purpose.

The new procedure for the separation of platelets, or more specifically of platelet-rich plasma (PRP), from whole blood is as follows: A new separation chamber (Fig. 1 and 2) is inserted into the Elutriator rotor and the Beckman system is primed with saline and purged of air. The rotor is then set on one side, without disconnecting its rotary seal from the exter-

nal flow system, and the chamber is carefully removed without letting the air into the flow system. The chamber is then emptied of saline and filled with AC anticoagulated whole human blood. The inlet and outlet ports are covered with a strip of sheet plastic which was prior to that smeared with silicone grease. The chamber is then inserted into the Elutriator rotor and the plastic strip pulled out. Covering the chamber's ports with the plastic strip prevents both spillage of blood and the entrance of air into the system. The centrifuge is set at 2,500 rpm and after one minute from the start of centrifugation elutriation at a low flow rate of 3.5 ml/min is initiated. It is necessary to hold the elutriation flow for one minute to clear the top of the separation chamber by partial sedimentation of red blood cells (RBCs) and white blood cells (WBCs) in order to prevent contamination of PRP. After two minutes of centrifugation, collection of platelets is started. The first 2 ml contains only platelet-poor plasma (PPP), while consecutively eluted 6 to 8 ml of PRP contains about 90% of the total platelets in blood.

Platelets collected by this procedure contain no WBCs and a very small number of RBCs. Functionally, platelets appear normal as based on their morphology, ability to aggregate, take up serotonin, and in their survival time in the blood circulation of animals. Their ability to secrete ATP during aggregation is on the average 32% higher than that of control platelets obtained by slow speed centrifugation, which suggests that they are even more functionally intact than the control platelets.

The apparatus shown in Fig. 3 and 4 represents a self-contained platelet separation system operating independently from the Beckman elutriator and used as an insert in any centrifuge with swinging buckets. The system incorporates a pump consisting of a piston 12 and a cylinder 13 which is operated by centrifugal force. Inside the piston 12 there is a conical chamber 14 with an inlet port 15 at its bottom and an outlet port 16 at its top. The o-ring 17 around the piston 12 provides the necessary seal with the cylinder 13. An annual groove 18 on the inner wall of the cylinder 13 serves to retain the o-ring 17 and to hold the plunger 12 in the upper position (Fig. 3) during the first minute of initial centrifugation at about 500 rpm. This provides the necessary delaying in the pumping action to allow partial sedimentation of blood in order to prevent contamination with blood of the upper collection chamber 19.

The platelet separation procedure using the device shown in Fig. 3 and 4 starts by filling the lower part of the cylinder 13 with 15 ml of saline through the inlet port 20 and then closing the valve 21. The conical chamber 14 is then filled with 21 ml of AC anticoagulated whole blood. The device is then placed into the swinging bucket of the centrifuge and spun at about 500 rpm for one minute to clear the upper part of the chamber from the blood cells. Thereafter, the centrfige speed is increased to about 2,000 rpm. At this higher centrifugal force the o-ring 17 snaps off the groove 18 and the plunger 12 begins to descend, thereby initiating pumping action. The flow rate is pre-adjusted with the needle valve 22 to about 5 ml/min so that in about 3 minutes all saline is pumped out from the cylinder (Fig. 4) into the separation chamber 14 and thereby displaceing all PRP from blood into the upper collection chamber 19. For the purpose of cleaning the collection chamber 19, it has screw arrangement and an o-ring 23 sealing it to the conical chamber 14.

Platelets collected with the system shown in Fig. 3 and 4 are even purer than those obtained by the previous procedure involving the use of elutriator rotor and the chamber shown in Fig. 1 and 2. In analyzing under a microscope more than 10^6 cells, not a single white or red blood cell has been found. This system also allows harvesting close to 90% of the total platelets in blood. Isolated by this system, platelets are just as functional as those isolated by the previous system (Fig. 1 and 2).

The apparatus for clinical use shown in Fig. 5 and Fig. 6 through Fig. 12 is a modification of the apparatus shown and described with reference to Figs. 3 and 4. It is designed as a disposable system consisting of three collapsible bags which are completely sealed and will allow one to maintain sterility during all the steps of the operation. Thus, the system is designed to comply with the FDA requirements of safety for human use. This system consists of a conical bag 1 functioning as a separation chamber which is held in a rigid support 2 split into two halves (Fig. 6) and having conical cavity. Below the conical bag 1 there is a satellite bag 3 attached which is filled with the elution medium such as normal saline. The satellite bag 3 is connected to the conical bag 1 by means of a short capillary tube 4 (Fig. 5 and 12) which is covered with a small ball 5 functioning as a ball valve. On a side of the satellite bag 3 there is a stopper arrangement 6 for the sterile infusion of medium into the bag 3. The rigid support 2 is also holding a collection bag 7 positioned above the conical bag 1. Both conical and collection bags have puncturing-type connector-arrangements 8 and 9 which allow aseptic connection and disconnection of these bags by means of a double-ended sterile needle connector 10. In order to hold the puncturing-type connector arrangement 8 in place there is an annular ring clamp 15 incorporated into the design of the rigid support 2 so that when the two halves are assembled the connector arrangement 8 is secured during subsequent operational steps. Both halves of the rigid support 2 are held together by an o-ring 11 which also functions as snap-ring fitting into the groove 12 on the inner wall of the centrifuge bucket 13 and holding the rigid support 2 in its upper position within the centrifuge bucket. The indented configuration 14 shown at the lower end of the rigid support 2 is provided to prevent pinching of the bag 3 when the support 2 slides down and squeezes the bag 3.

The operational steps used in the separation of platelets with this system are illustrated in a diagrammatic series which is represented in Fig. 6 through Fig. 12. Figure 6 shows a vertical sectional view of the device in unassembled form. All three bags, 3, 1 and 7, are shown empty and collapsed with the upper collection bag 7 being disconnected. In Fig. 7 is shown priming of the conical bag 1 with saline and PPP. The conical bag 1 is shown inserted

in one-half of the rigid support 2. Figure 8 shows an assembled system positioned inside the centrifugal bucket 13. The o-ring 11 which holds together both halves of the support 2 also holds the assembled support in the upper position of the centrifuge bucket where the o-ring 11 snaps into the groove 12 in the bucket 13. This holding arrangement allows one to subject the device to slow speed centrifugation, for instance at 500 rpm, for about one minute in order to clear the top of the chamber from blood cells. Thereafter the centrifugation speed is increased to about 2,000 rpm at which the o-ring 11 is snapped off the groove 12 and the support 2 begins to slide down against the satellite bag 3, which forces saline through the capillary tube 4 into the conical bag 1. This action displaces PRP from the blood sample into the upper collection bag 7 as is illustrated in Fig. 9. Thereafter the collection bag 7 is disconnected, placed into a smaller bucket of another centrifuge (Fig. 10), and centrifuged at high speed in order to obtain PC and PPP. PPP is then mixed with the blood remaining in the conical bag 1 (Fig. 11), and blood containing both saline and PPP is reinfused into the donor. Fig. 12 shows an enlarged sectional view of the capillary connector and the ball valve between the satellite bag 3 and conical bag 1.

The apparatus shown in Fig. 13 and 14 is a version of a scaled-up system represented in Fig. 5 but incorporating only one collapsible bag 3 containing saline and having a conical separation chamber 17 housed inside the cylindrical body 18 which can move under centrifugal force inside the centrifuge bucket 13 and exerts pressure against the collapsible bag 3. The PRP receiving chamber 19 is sealed with the o-ring 20 to the cylindrical body 18 above the separation chamber 17. The device also has a needle valve 21 (Fig. 14) for the regulation of flow rate incorporated inside a modular insert 22 and having a nipple for connecting the collapsible bag 3.

Figure 15 is a modular insert representing a possible version of an arrangement for delaying liquid flow during the start of centrifugation until maximal centrifugal force activates valve 23. It also incorporates needle valve 21 for controlling flow rate.

In the method described above using one of the three forms of apparatus for separating platelets from a blood sample, the body of saline has a volume between 0.2 and two times the volume of plasma in the blood sample and flows during centrifuging at a rate less than about 1.0 ml per minute per ml of blood sample.

CLAIMS

1. Apparatus for separating solid particles such as platelets from a liquid sample such as blood comprising a body adapted to be subjected to centrifugal force and containing a cavity adapted to receive a volume of displacing liquid therein, a piston mounted in the body for movement into the cavity in response to centrifugal force on the piston with the piston containing a centrifugal chamber for containing a sample from which particles are to be separated, and having inner and outer ends, and injection passageway means to allow movement of displacing liquid from the cavity to the centrifugal outer end of the chamber in response to movement of the piston into the cavity, a discharge passageway at the centrifugally inner end of the chamber for discharging particle rich liquid in response to injection of displacing liquid into the chamber, and means for preventing flow of displacing liquid from cavity to the chamber before centrifugal stratification of blood cells in the chamber.

2. Apparatus as claimed in Claim 1 including control means for preventing movement of the piston into the cavity until a minimum centrifugal force is applied to the piston.

3. Apparatus as claimed in Claim 2 in which said control means comprises an O-ring on the piston received in a groove in the wall of the cavity.

4. Apparatus as claimed in Claim 2 in which the control means comprises valve means activated by centrifugal force in said injection passageway means.

5. Apparatus as claimed in any one of Claims 1 to 4 including valve means in said injection passageway means for controlling the flow rate therein.

6. Apparatus as claimed in any one of Claims 1 to 5 including a blood sample in said chamber and a volume of buffered saline in the cavity.

7. Apparatus as claimed in Claim 6 in which the body of saline has a volume between 0.2 to two times the volume of plasma in the blood sample.

8. Apparatus as claimed in any one of Claims 1 to 7 further comprising a collapsible bag in the cavity surrounding the body of displacing liquid and communicating with the injection passageway means.

9. Apparatus as claimed in Claim 8 including a second bag for containing a blood sample in the chamber and communicating with the collapsible bag in the cavity, and a third bag communicating through the discharge passageway with the second bag for receipt of platelet rich plasma.

10. A triple bag adapted to be received in a centrifugal apparatus for separating platelet rich plasma from a blood sample comprising: a central portion having a generally conical shape with a centrifugally outer end at the vertex of the cone and a centrifugally inner end at the opposite end thereof and adapted to contain the blood sample, a collapsible injection portion adapted to contain a volume of saline less than the volume of the central portion of the bag and communicating with the centrifugally outer end of the central portion for injecting saline into the central portion as the injection portion collapses, and an expandable collection portion communicating with the centrifugally inner end of the central portion for collecting platelet rich plasma displaced from the central portion.

11. A method of separating platelets from a blood sample comprising: supporting the blood sample in a centrifugal chamber having inner and outer ends, subjecting a chamber to an initial centrifugal force of at least a predetermined minimum to stratify red cells in the sample away from the inner end, and subjecting the chamber to a second centrifugal force while injecting into the outer end of the chamber from a cavity a displacing liquid having a volume of between 0.2 and two times the volume of plasma in the sample at a flow rate less than about 1.0 ml. per minute per ml. of the blood sample, and

displacing platelet rich plasma from the inner end of the chamber at substantially the same volume and rate, with the displacing liquid ejected from the cavity by the second centrifugal force on the blood sample.

12. The method of Claim 11 in which said chamber has a generally conical cross-section and said displacing liquid is buffered saline, and the chamber is subjected to a greater centrifugal force during the injection than during the stratification.

13. The method of Claim 11 in which said displacing liquid is injected at a volume approximately equal to volume of plasma in the sample and at a rate of about 0.35 ml. per minute per ml. of the sample.

14. The method of Claim 11 in which the step of injecting the displacing liquid into the chamber is performed by impounding the displacing liquid in a support cavity and propelling the blood sample containing chamber into the cavity under the influence of centrifugal force.

15. The method of Claim 11 in which a sufficient number of blood cells are present in the chamber in order to form a dense cell suspension which has characteristics of a fluidized bed of particles and acts as a depth filter allowing platelets to pass through freely while maintaining other blood cells in a steady state equilibrium in the chamber.

Printed for Her Majesty's Stationery Office by The Tweeddale Press Ltd., Berwick-upon-Tweed, 1981.
Published at the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.